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Original Article

Low Serum Concentrations of Vitamin B6 and Iron Are Related to Panic Attack and Hyperventilation Attack

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Patients undergoing a panic attack (PA) or a hyperventilation attack (HVA) are sometimes admitted to emergency departments (EDs). Reduced serotonin level is known as one of the causes of PA and HVA. Serotonin is synthesized from tryptophan. For the synthesis of serotonin, vitamin B6 (Vit B6) and iron play important roles as cofactors. To clarify the pathophysiology of PA and HVA, we investigated the serum levels of vitamins B2, B6, and B12 and iron in patients with PA or HVA attending an ED. We measured each parameter in 21 PA or HVA patients and compared the values with those from 20 volunteers. We found that both Vit B6 and iron levels were significantly lower in the PA/HVA group than in the volunteer group. There was no significant difference in the serum levels of vitamins B2 or B12. These results suggest that low serum concentrations of Vit B6 and iron are involved in PA and HVA. Further studies are needed to clarify the mechanisms involved in such differences.

Key words: panic, hyperventilation, vitamin B6, iron, serotonin

Panic attack (PA) and hyperventilation attack (HVA) are paroxysmal psychiatric events, and the symptoms occur suddenly. Individuals who are undergoing a PA or HVA are sometimes taken by ambulance to the emergency department (ED) of a hospital because of the physical and mental symptoms (including a feeling of fear), and these individuals often experience repeated attacks [1, 2]. Radical treatment is sometimes needed for individuals who experience repeated PAs and/or HVAs. It is necessary to clarify the pathophysiology of PA and HVA and to establish valid means of treatment.

Miller *et al.* [3] demonstrated that a reduction of the brain serotonin level in patients experiencing a PA accelerated the attack, while Olson *et al.* [4] reported, in preclinical studies using rats, that the reduction of brain serotonin level induced hyperventilation. Thus, several studies have suggested the involvement of a serotonin deficiency in the etiology of PA and HVA [3–6]. Serotonin is synthesized from tryptophan, and it is well known that vitamin B6 (Vit B6) and iron each play an important role as cofactors in the synthesis of serotonin [7, 8]. Low serum concentrations of Vit B6 or iron have been reported as factors related to a reduction in the brain level of serotonin [9–11]. However, no study analyzing the relationship of PA and HVA onset to serum concentrations of Vit B6 and iron has been reported, to the best of our knowledge.

The present prospective observational study compared the serum levels of Vit B and iron in patients visiting the ED because of a paroxysmal psychiatric disorder (PA or HVA) with those in healthy volunteers. The purpose of the study was to investigate whether Vit B6 and iron are related to the develop-

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ment of PA or HVA.

Materials and Methods

We analyzed serum levels of vitamin B2 (Vit B2), Vit B6, vitamin B12 (Vit B12), hemoglobin (Hb) and iron in premenopausal women diagnosed as having a PA or HVA who visited the ED of Atago Hospital (Kochi Prefecture, Japan), and in healthy volunteers. The study was approved in advance by the Ethics Committee of Atago Hospital, and informed consent was obtained from each participant prior to the start of the study. Atago Hospital is a 562-bed general hospital that accepts approx. 7,800 patients requiring critical care each year.

Participants. The attack group (AT) was composed of premenopausal women diagnosed as having a PA or HVA among all patients who visited the hospital's ED during the 1-year period from November 2010 to October 2011. The diagnosis of PA was based on the Diagnostic and Statistical Manual of Mental Disorders IV text revision (DSM-IVTR). HVA was diagnosed based on tachypnea (other than PA); tachypnea is characterized by excessive ventilation beyond the demands from physical metabolism, and is induced by psychogenic causes (stress, fear, anxiety or tension) in individuals free of physical abnormalities [12]. For patients who were minors, an explanation about the study was given to the patient's guardian, and the guardian's consent was obtained before blood sampling and tests. Patients who did not issue consent to the study, patients with hepatic disease (alanine transaminase > 44 U/l), patients with kidney disease (serum creatinine > 0.01 mg/ml), patients with endocrine or inflammatory disease, and patients routinely taking food supplements were excluded from this study.

The control group (Co) was composed of employees of the hospital or their family members who satisfied the following requirements: premenopausal women who gave consent to blood sampling for research purposes during a periodic health checkup in November 2010 and who were free of psychiatric symptoms, autonomic nervous symptoms and headache. Like the AT group, individuals with hepatic disease, kidney disease, endocrine disease or inflammatory disease, and individuals routinely taking food supplements were excluded from the Co group.

Measurements. General emergency tests and the measurement of serum Vit B2, Vit B6, Vit B12 and iron were carried out during visits to the ED of Atago Hospital (AT group) or during the periodic health checkup (Co group). The Co group subjects fasted after having dinner the previous night, and blood was collected the next morning at 9 : 00 a.m. The methods employed for measurement were the lumiflavin fluorescence method for Vit B2, high-performance liquid chromatography (HPLC) for Vit B6, a chemiluminescent immunoassay for Vit B12 [13] (BML Inc., Tokyo), and colorimetry for serum iron [14].

The serum Vit B6 measurement included measurements of pyridoxamine (PAM), pyridoxal (PAL), and pyridoxine (PIN). The levels of 3 phosphonate forms of vitamers detected in the serum, namely, pyridoxamine 5'-phosphate, pyridoxal 5'-phosphate (PLP), and pyridoxine 5'-phosphate were measured after conversion into PAM, PAL, and PIN, respectively. PAM and PIN levels were less than the quantitation limit in all participants. Therefore, the Vit B6 data used in this study pertained completely to PAL levels.

Statistical analysis. Statistical data are expressed as median and quartiles. A Mann-Whitney U test was employed for comparisons between the AT group and the Co group. The magnitude of association between each factor and attack onset was evaluated using the area under a receiver operator characteristic (ROC) curve. To analyze the association of each factor with the independent outbreak of an attack, a multivariate logistic regression analysis was carried out, with factors with a p-value of 0.1 or lower serving as independent variables and the outbreak of attack as a dependent variable. Mutual associations among factors were evaluated by variance inflation factors (VIFs).

It was assumed that the standard deviation of Vit B6 was 11 ng/ml and that a difference of 10 ng/ml was a clinically significant difference. Therefore, it was necessary to collect data from 20 subjects in each group to achieve an alpha of 0.5 and a power of 0.8. A p-value less than 0.05 was regarded as significant. The statistical analyses were carried out with the computer program SPSS 20.0 (IBM, Chicago, IL, USA).

Results

During the study period, 7,734 patients visited the Atago Hospital ED. Of these patients, 44 premenopausal women were diagnosed as having a PA or a HVA. Of these patients, 21 gave informed consent and participated in the study (the AT group). Twenty volunteers gave written consent after being fully informed of the study design and were enrolled in the study (the Co group).

Background variables. Table 1 shows each factor in the AT group and Co group. In the AT group (n = 21), 10 patients were diagnosed with PA and 11 were diagnosed with HVA. The age did not differ significantly between the 2 groups (AT vs. Co) ($p = 0.1$). In the AT group, one patient was taking oral contraception, one patient was using oral benzodiazepine (BZO), one was using a selective serotonin reuptake inhibitor (SSRI) and oral BZO, and one was taking a serotonin-norepinephrine reuptake inhibitor (SNRI) with a multi-acting receptor targeted antipsychotic (MARTA), sodium valproate and oral BZO.

Hb, Iron, Vit B2, Vit B6, and Vit B12 variables. Hb did not differ significantly ($p = 0.45$, Table 1) between the AT group (132 mg/ml) and the Co group (132 mg/ml). The iron level was significantly lower ($p < 0.001$) in the AT group (0.46 μg/ml) than in the Co group (0.98 μg/ml) (reference value [RV]: 0.56–1.39 μg/ml). The Vit B6 level was also significantly lower ($p = 0.002$) in the AT group (6.3 ng/ml) than in the Co group (12.8 ng/ml) (RV: 4.0–19.0 ng/ml). However, there was no significant difference between the 2 groups in terms of the Vit B2 level (AT group, 0.023 μg/ml; Co group, 0.025 μg/ml; RV: 0.017–0.046 μg/ml, $p = 0.95$) or the Vit B12 level (AT group, 428 pg/ml; Co group, 468 pg/ml; RV: 233–914 pg/ml, $p = 0.64$) (Fig. 1).

Table 2 shows the area under the ROC curve indicating the involvement of each factor in the onset of PA and HVA. The areas under the ROC curve for serum iron and Vit B6 were 0.82 and 0.86, respectively, indicating the close association of these 2 factors with PA and HVA.

Multivariate analysis. Three factors (age, iron and Vit B6) had a *p*-value below 0.1. These 3 factors were thus adopted as independent variables for a

Table 1 Age, hemoglobin, iron and vitamin B levels in the AT group and Co group

	AT group n = 21	Co group n = 20	*p*-value
Age	26 (21, 37)	34 (29, 41)	0.1
Hb (mg/ml)	132 (124, 138)	132 (128, 140)	0.45
Iron (μg/ml)	0.46 (0.29, 0.72)	0.98 (0.78, 1.13)	<0.001
Vit B6 (ng/ml)	6.3 (4.5, 8.7)	12.8 (8.9, 25.8)	0.002
Vit B2 (μg/ml)	0.023 (0.022, 0.028)	0.025 (0.022, 0.028)	0.95
Vit B12 (pg/ml)	428 (315, 635)	468 (382, 623)	0.64

Data shown include the median (25th percentile, 75th percentile) and *p*-value. The iron and Vit B6 levels were significantly lower in the AT group than in the Co group. Other parameters were not significantly different between the 2 groups.

Table 2 Area under receiver operator characteristic curve for each factor (Hb, iron, Vit B)

		95% confidential interval (CI)	
	Area under ROC curve	Lower	Upper
Age	0.64	0.46	0.82
Hb	0.60	0.42	0.77
Iron	0.82	0.69	0.96
Vit B6	0.86	0.74	0.97
Vit B2	0.55	0.37	0.73
Vit B12	0.61	0.43	0.80

ROC: receiver operator characteristic.

Table 3 Multivariate analysis of association of age, iron, and Vit B6 with attacks

	Odds Ratio (95% CI)	*p*-value	VIF
Age	0.99 (0.92–1.08)	0.89	1.11
Iron	0.96 (0.94–0.99)	0.006	1.14
Vit B6	0.90 (0.82–0.99)	0.037	1.05

VIF: Variance inflation factor. A multivariate analysis was carried out using 3 factors (age, iron and Vit B6) as independent factors. Iron and Vit B6 were identified as independent factors associated with the onset of PA or HVA.

multivariate analysis (Table 3). Iron (adjusted odds ratio = 0.96, $p = 0.006$) and Vit B6 (adjusted odds ratio = 0.90, $p = 0.037$) were independently associated with the onset of PAs or HVAs. The VIF of each factor was less than 5, and there was no multicollinearity among the factors. For this model, the Hosmer-Lemeshow test resulted in a p-value of 0.47.

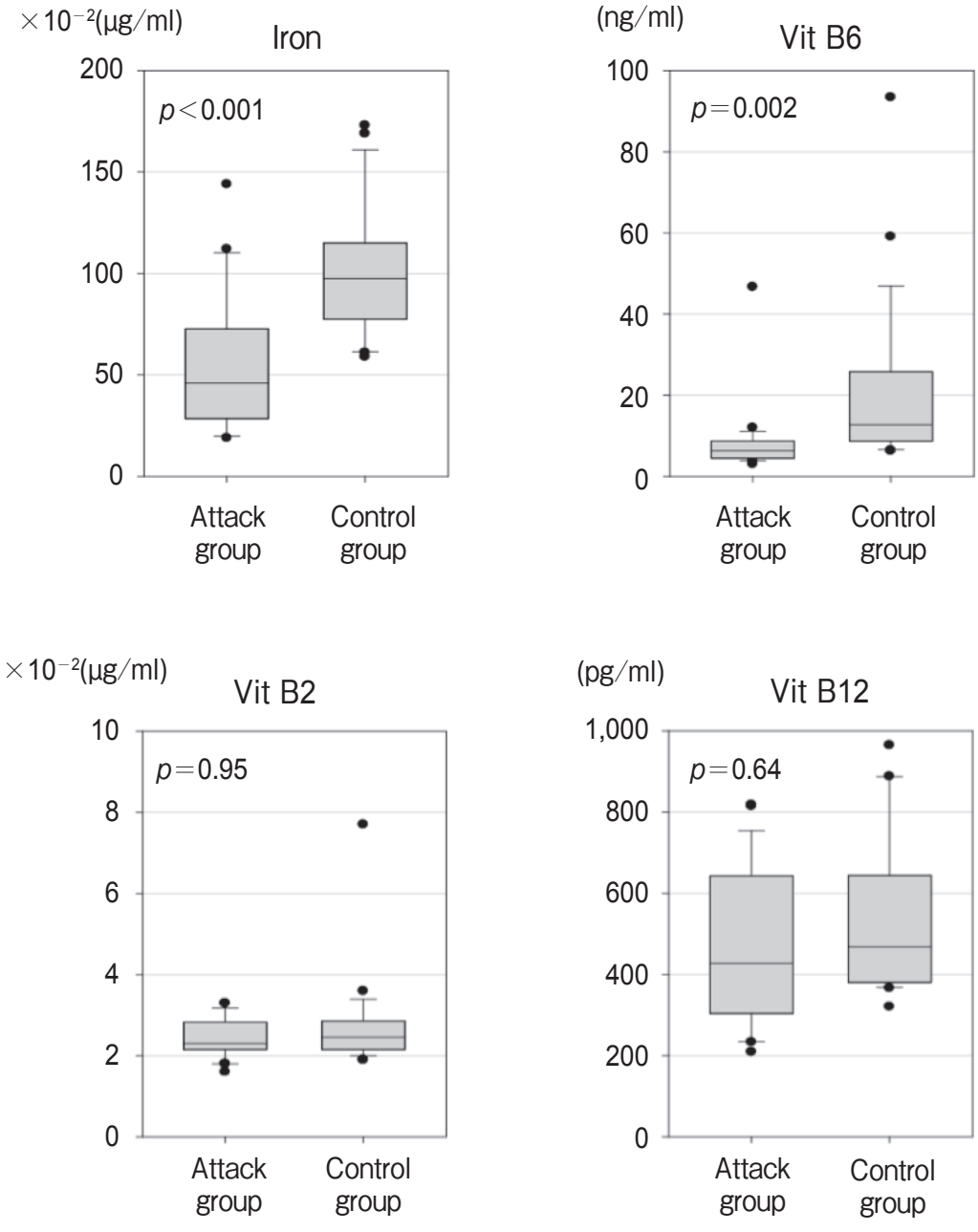

Fig. 1 Comparison of iron, Vit B6, Vit B2, and Vit B12 levels between the AT group and Co group. Results of Mann-Whitney U tests on serum iron, Vit B6, Vit B2 and Vit B12 between the AT group and Co group. Iron (0.46 μg/ml and 0.98 μg/ml, $p < 0.001$) and Vit B6 (6.3 ng/ml and 12.8 ng/ml, $p = 0.002$) were significantly lower in the AT group. There was no significant intergroup difference in terms of Vit B2 (0.023 μg/ml and 0.025 μg/ml, $p = 0.95$) or Vit B12 (428 pg/ml and 468 pg/ml, $p = 0.64$).

Discussion

In the present study, patients who attended the ED and were diagnosed with PA or HVA provided measurements of Hb, Vit B2, Vit B6, Vit B12 and iron levels, and we compared these measurements with data from healthy volunteers. The results revealed significantly lower serum Vit B6 and iron levels in the patients with PA or HVA, suggesting an association of Vit B6 and iron deficiency with the onset of PA and HVA. There was no significant difference in the Vit B2 or Vit B12 levels. Hb also did not differ significantly between the AT and Co groups. Thus, the influence of the symptoms of anemia arising from iron deficiency was ruled out.

PA and HVA are clinically considered as psychiatric disorders triggered by factors such as anxiety, stress, or depression. However, the exact cause or causes of the onset of a PA or HVA have not yet been identified. From a neurobiological perspective, serotonin deficiency and dysfunction of serotonin neurotransmission have been suggested as factors commonly involved in the etiology of PA and HVA [3–6]. Serotonin is synthesized from the precursor tryptophan. In the serotonin synthesis system of the brain, Vit B6 is a coenzyme for tryptophan hydroxylase, which is involved in the conversion of tryptophan into 5-hydroxytryptophan [7], and iron serves as a cofactor for aromatic L-amino acid decarboxylase involved in the formation of serotonin from 5-hydroxytryptophan [8]. Therefore, a reduction in Vit B6 and iron levels can suppress the progression of the serotonin synthesis.

Bell *et al.* [5] reported serotonin deficiency and dysfunction of serotonin neurotransmission as causes of PA, because serotonin at the periaqueductal gray matter (PAG) suppresses PA. Hoes *et al.* [6] also considered compromised serotonergic neurotransmission as a cause of HVA. In addition, since PA and HVA have common features in terms of symptoms, pathophysiology and compromised serotonergic nerve function [15, 16] in PAG associated with the host defense system [17, 18], we suspect that PA and HVA belong to the same serotonin-related spectrum of psychiatric disorders. In the present study, PA and HVA were analyzed together in a single group (AT group).

For patients with depression, a low brain serotonin level is known to be a contributing factor [19], and an association between depression and low serum Vit B6 levels and iron deficiency has also been reported [20–23]. These reports suggest that a serotonin level reduction arising from Vit B6 deficiency [11] or iron deficiency [24] leads to symptoms such as those of depression [22, 23]. The present study did not involve measurement of serotonin levels in the blood or the cerebrospinal fluid, and thus we do not know whether a reduction in serotonin level resembling that seen in patients with depression existed in the PA/HVA patients.

For the measurement of Vit B6, serum PLP evaluation is often used. In the present study, however, we adopted the method of measuring PAL after conversion from PLP because we usually measure PAL in our clinical department. Analyses of Vit B levels should take into account circadian variations and the influence of a meal consumed shortly before measurement. The AT group was allowed to take meals freely, whereas the Co group fasted for 12 h or more before their measurements were taken. The Vit B levels would therefore be expected to be lower in the Co group than in the AT group. Interestingly, however, the Vit B6 levels were lower in the AT group than in the Co group.

Moreover, we could not determine whether the reductions in Vit B6 and iron levels were secondary to the PA and HVA, or were the primary cause. The possible reasons for a reduction in Vit B6 and iron levels include a decrease in the intake of Vit B6 and iron due to illness, and the influence of medication. However, regarding a decrease in intake due to illness, the AT and Co patients' Vit B2 and Vit B12 levels showed no reduction. This suggests that an extreme shortage of nutrient intake is unlikely in patients with PA or HVA. Regarding of medication, only four AT patients were taking medications. Therefore, the influence of medication seemed to be low in the present study.

We could not evaluate the severity of depression in the patients diagnosed as having a PA or HVA, because the patients tended to be mentally unstable at the time of their visits to the ED. It is an open question whether the low Vit B6 and iron levels in patients with PA or HVA is attributable to depression or to the PA or HVA.

In addition, although 44 women were diagnosed as

having PA or HVA, only 21 of these women participated in the study (AT group). Therefore, patient selection biases also need to be taken into consideration. Another limitation of this study was the fact that the study was carried out at a single facility. Although this study was based on a power analysis, its scale was small and the possibility of a Type 1 error is not negligible.

In conclusion, patients admitted to the emergency department of a hospital as they were undergoing a panic attack or a hyperventilation attack had significantly lower serum Vit B6 and iron levels compared to healthy volunteers. Further studies examining the effects of a therapeutic intervention to elucidate how low serum Vit B6 and low serum iron are involved in the pathophysiology of panic attacks and hyperventilation attacks are necessary to explore the results of the present study.

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